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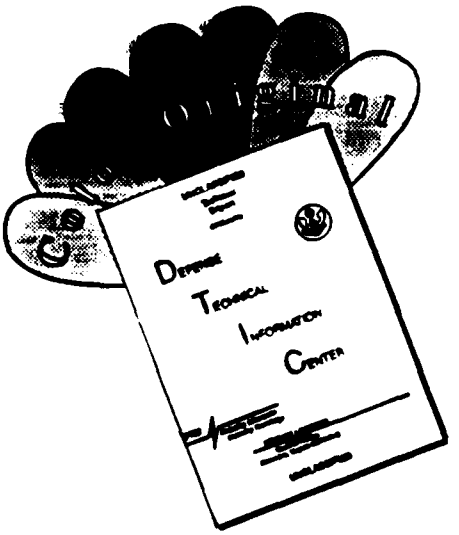

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Considering the demonstrated benefits, it may seem rhetorical to ask why ACE inhibitors are so popular. I believe that their widespread use has come about because of a far deeper and better understanding of the basic science involved. Few clinical cardiologists knew about the renin-angiotensin system 20 years ago. Now the role of this system in circulatory control is understood by almost all.

The current emphasis has swung to the role of angiotensin II as a growth factor, helping to regulate early stages of protein synthesis pathways. It is clear that more and more attention will be given to the cellular mechanisms underlying myocardial and vascular growth, with the object of achieving optimal remodeling in response to enhanced intraventricular or intravascular mechanical stress. Likewise, the harmful effects of hypertension on the intravascular pressure within the renal glomeruli will come to be better understood, especially in the case of hypertension combined with diabetes.

Our understanding of the actions of the ACE inhibitors advances along with the molecular biology because trials in patients with vascular and left ventricular hypertrophy have kept pace. Academic and pharmaceutical researchers have worked closely together for the advance of knowledge and the ultimate benefit of the patient. I think it is these concordant developments that have made these agents winners.

Prophecies are often wrong; but here are mine. First, combination therapy with an ACE inhibitor and a diuretic is going to become standard first-line treatment for hypertension and for heart failure. Diuretic therapy is already first-line for both of these conditions, low doses for hypertension and higher doses for heart failure. By virtue of the therapeutic sodium loss, however, diuretic therapy induces release of plasma renin from the kidneys and increases circulating vasoconstrictive angiotensin II, an adverse development for both hypertension and cardiac failure. Hence the logic of the combination.

Secondly, at a molecular level, there will be a more precise understanding of the role of local tissue renin-angiotensin systems, exactly what they do and how they can be controlled. The link between these systems and proto-oncogenes will become apparent. ACE inhibitors will be combined with other modalities to adequately control unwanted protein synthesis.

Thirdly, the potential protective role of the ACE inhibitors on the vascular endothelium and against infarction will lead to better comprehension of the endothelium as a site for therapeutic drug action. After further research on the messengers involved in the protective effect of the ACE inhibitors, these agents will in my opinion have a far better understood role in preventing the vascular complications of hypertension, heart failure, and diabetes.

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Strategies for the Control of Malaria

by G. Thomas Strickland and Stephen L. Hoffman

The most virulent of the four malaria parasite species that infect humans is resistant to drugs in most endemic areas. Eradication of the mosquito vectors with residual insecticides is not a practical possibility. Using insect repellants, screening, and bed nets to prevent transmission is safe and effective but far from universal. Development of new drugs continues, along with attempts to engineer genetically modified mosquitoes. Although development of vaccines against the antigenically complex parasite is a difficult task, recent successful trials give hope that immunization will provide another weapon in the battle to control malaria.

Forty years ago, encouraged by the potency of residual insecticides such as DDT against malaria's mosquito vectors, the World Health Organization undertook a major campaign to eradicate the disease. Yet malaria today causes more human misery than ever before. Mosquitoes developed resistance to insecticides, and parasites to drugs. Poverty, ignorance, social disruptions, and often inadequate national resources for malaria control (and for other preventive health efforts) hamper what might otherwise be effective measures.

Malaria has a massive impact in sub-Saharan Africa, where it is the leading killer of children under 5. It is a major problem in southern Asia and western Oceania; in parts of Southeast Asia *Plasmodium falciparum* infections are resistant to all commonly used drugs. In much of South and Central America and in the Caribbean malaria threatens millions, particularly in Brazil, where mosquito-infested rain forests are being cleared and developed.

Published estimates are that malaria parasites annually infect 250 to 300 million people, cause 100 to 120 million clinical cases, and kill 1 to 2 million people. Recent work in sub-Saharan Africa suggests that such estimates are significantly low and that there may be 500 to 900 million clinical cases of malaria per year.

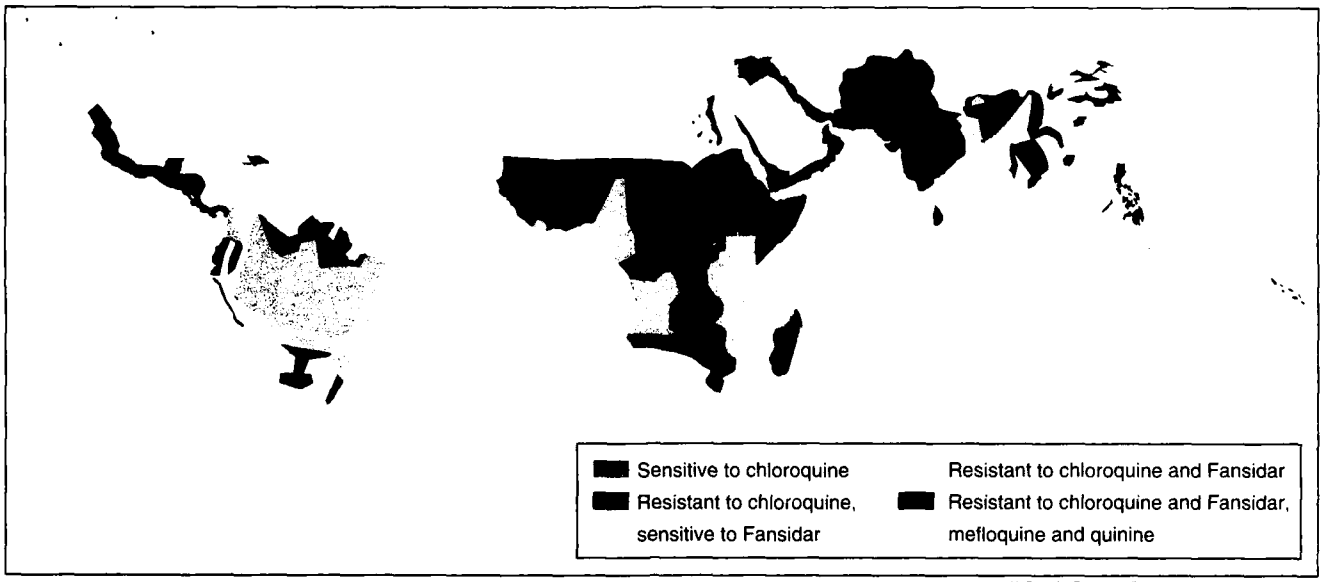
Mortality is just as difficult to determine. Young children, who are still developing protective immunity, and primigravid women are the most susceptible. Children in endemic areas who die of malaria often have diarrhea, measles, malnutrition, or severe anemia that may be considered the cause of death. It is reasonable to suppose that malaria contributes to 3 to 4 million deaths a year worldwide.

Strategies Differ Among Populations

Strategies for malaria control in nonimmune individuals who travel to areas where malaria is endemic differ from control of the disease in semi-immune populations. Travelers from developed countries are liable to severe infections, and at least 10 to 20% of them, having no immunity to *P. falciparum*, will die if not diagnosed or if inappropriately treated.

Among travelers to malarious areas, the most important single preventive measure is chemoprophylaxis. However, the risk of developing malaria can be significantly reduced by limiting exposure to the anopheline mosquitoes that transmit the infection. Staying in screened areas from dusk to dawn, wearing protective clothing, using insect repellants, and sleeping under bed nets are all partially effective. If the prophylactic drugs and other measures fail, the traveler must receive prompt treatment with an antimalarial drug.

In endemic areas where transmission of malaria is low, and thus



IAN WORPOLE; DATA FROM HANS O. LOBEL, M.D.

Malaria is a tropical disease. Its endemic regions are in *color*. The most difficult problems with drug resistance occur along the borders of Thailand, where the parasites are resistant to mefloquine as well as to chloroquine and Fansidar (pyrimethamine-sulfadoxine).

where most of the population is susceptible to infection, such as urban and peri-urban districts in Asia and Latin America, the goal is also to prevent infection. Rapid diagnosis and treatment of clinical cases controls disease and reduces transmission; eliminating breeding sites or treating them with insecticides controls the mosquito vectors.

Where mosquitoes rest indoors during the day, residual insecticides used just before and during the major transmission season may still be cost-effective, though repeated applications are required, raising costs and environmental concerns. Individual efforts to reduce vector-human contact are inexpensive. Chemoprophylaxis in most cases would be excessive for the amount of disease prevented.

It is not now feasible to interrupt transmission where vectors are abundant and effective, as in sub-Saharan Africa, where 80% of the world's malaria infections occur and virtually everyone is infected early in life. Treatment of symptoms, following a confirmed diagnosis if possible, is the most important measure. Chemoprophylaxis for young children and pregnant women reduces morbidity and mortality. Community-wide use of insecticide-impregnated bed nets can significantly reduce mortality.

Vector Control Is Definitive but Difficult

Of almost 500 species of anopheline mosquitoes, about 30 are important transmitters of malaria. Chemical compounds that kill egg, larval, and pupal stages are suitable only where breeding sites are few and areas involved are limited.

The female mosquito needs a blood meal every second or third night for successful egg laying. The shortest intrinsic cycle of the parasite in the mosquito is eight days, so at least one intermediate feeding is necessary to transmit malaria from an infected to a susceptible human.

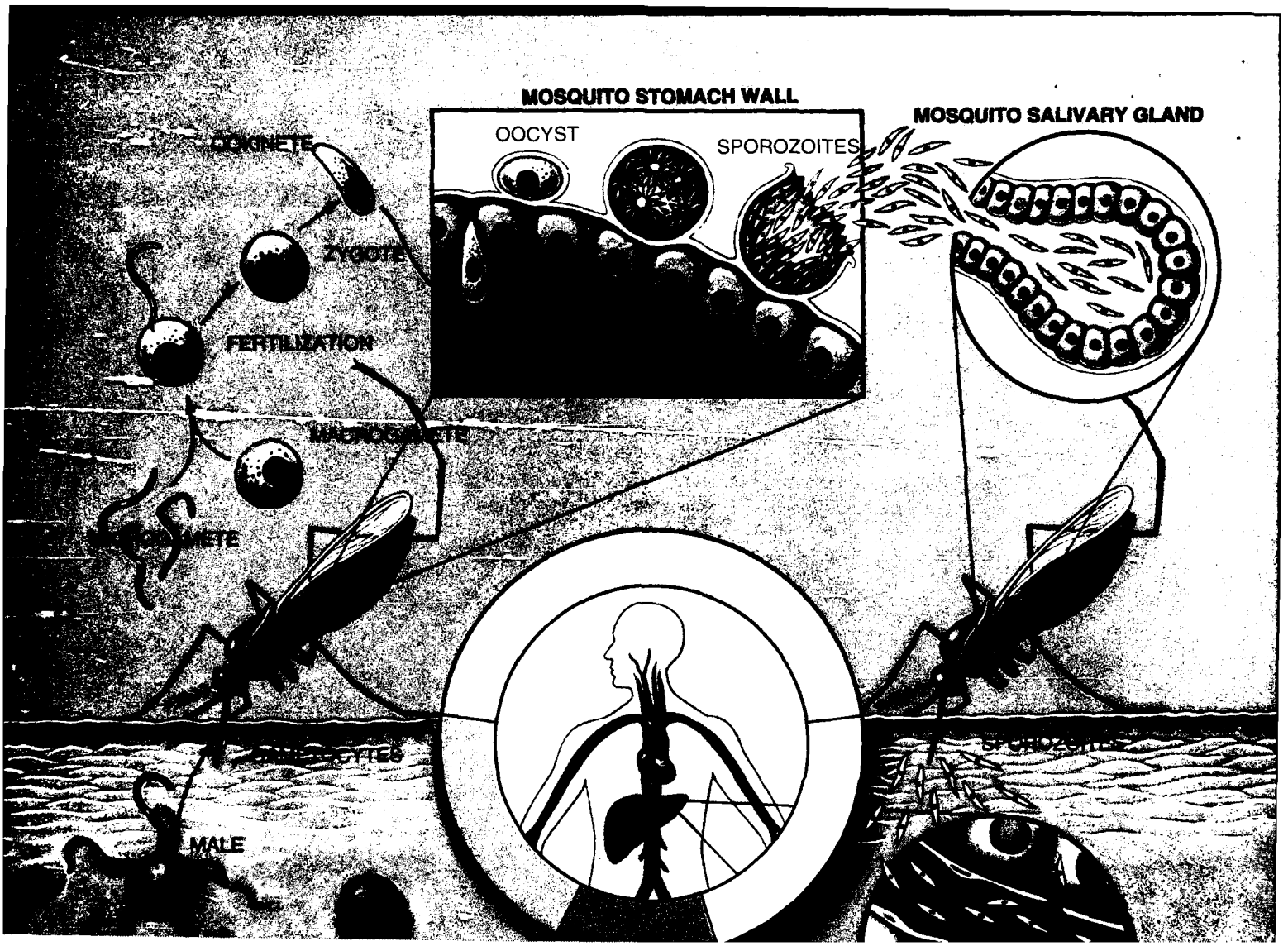
A practical way to reduce the risk of infection is to limit potential contact with mosquitoes, which are active from dusk to dawn. Insect repellants (DEET), insecticides (pyrethrins), and bed netting are effective, and impregnating mosquito nets with pyrethrin adds further protection. Treated nets reduce the number of mosquitoes entering a room where they are used and kill those that land on the nets.

A large trial carried out in The Gambia by Brian Greenwood and his associates concluded that impregnated bed nets reduced child mortality and could be introduced readily in a primary health care program. Adding chemoprophy-

G. THOMAS STRICKLAND directs the International Health Program in the Department of Epidemiology and Preventive Medicine at the University of Maryland School of Medicine and is the editor of a textbook of tropical medicine.

STEPHEN L. HOFFMAN directs the malaria program at the Naval Medical Research Institute and recently won the Bailey K. Ashford Medal, given by the American Society of Tropical Medicine and Hygiene to outstanding young investigators in tropical medicine.

Frank H. Collins of the Division of Parasitic Diseases, CDC, reviewed and revised the section on vector control. James M. Crutcher of the Naval Medical Research Program contributed the section on chemotherapeutic drugs.


MOSQUITO STOMACH WALL
OOCYST
SPOROZOITES
MOSQUITO SALIVARY GLAND
ZYGOTE
FERTILIZATION
MALE

THE COMPLICATED LIFE CYCLE OF THE MALARIA PARASITE

(ILLUSTRATION ON THE OPPOSITE PAGE)

The sexual stage of the parasite's life cycle takes place in the mosquito, which is its definitive host. Sporogony occurs in the mosquito's midgut following the ingestion of gametocytes from an infected human. The microgametocyte exflagellates to produce four to eight microgametes, each capable of fertilizing a macrogamete to form a zygote, which elongates to become a motile ookinete. These force their way through and between the epithelial cells to the outer surface of the midgut, where they round up into spheres called oocysts. An infected mosquito usually has one to ten oocysts.

The oocyst nucleus divides repeatedly while the oocyst enlarges. Each divided nucleus forms an elongated fusiform sporozoite, and these are released by the thousands into the body cavity of the mosquito when the oocyst eventually ruptures. The mosquito becomes infectious when sporozoites migrate to its salivary glands. This intrinsic cycle usually takes 8 to 20 days.

Injection of sporozoites into the human bloodstream by a feeding mosquito is followed within minutes by their disappearance from the blood. Those that survive enter the parenchymal cells of the liver and multiply asexually, forming thousands of uninucleate merozoites within a schizont. After 6 to 16 days the hepatic schizont ruptures, releasing merozoites into the circulation, where they invade erythrocytes.

The first erythrocytic stage is a small, rounded trophozoite called a ring form. These feed on hemoglobin, growing and then differentiating to erythrocytic schizonts that release merozoites into the circulation when the infected red cell ruptures, and these merozoites infect other erythrocytes.

Some merozoites released from ruptured erythrocytes develop into female macrogametocytes and male microgametocytes. These are not associated with clinical illness but infect feeding mosquitoes. What induces the appearance of the sexual stages is not known.

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laxis reduced the incidence of clinical malaria but did not further improve survival. The estimated costs per child-year were comparable to those for measles vaccination and oral rehydration fluids, two successful public health interventions in developing countries.

The most important vector of malaria parasites in Africa is the *Anopheles gambiae* mosquito. In 1986 Frank H. Collins of the CDC described a genetically selected strain of this mosquito that encapsulated and killed *Plasmodium* ookinetes in a melanin capsule. This observation invited consideration of the use of genetic engineering to introduce such parasite-killing genetic traits into the natural vector population as a strategy for malaria control. In collaboration with Fotis Kafatos of Harvard's Department of Cellular and Developmental Biology, research was initiated to develop a high-resolution genetic map of the three *Anopheles gambiae* chromosomes that could be used to identify and clone the gene or genes involved in the encapsulation response. The first stage of this map, covering the X chromosome, has been published.

Genes from organisms other than the mosquito might also be used to block normal parasite development. An approach being pursued by Robert Sinden of Imperial College and his colleagues in London and Rome is the use of a mammalian ookinete-specific antibody that had already been shown to block parasite development when included in the parasite-containing mosquito blood meal.

Delivery of parasite-specific antibody genes or parasite encapsulation genes into the mosquito genome will require development of the appropriate tools for genetically engineering the mosquito. Movement of such genes into the natural population will also be a problem. The currently favored solutions involve the use of infectious transposable elements or obligate symbiotic organisms as vehicles

AGENTS OF HUMAN MALARIA

Plasmodium falciparum
"Malignant tertian malaria"
Most dangerous; sludging of erythrocytes can obstruct the microcirculation; many strains are resistant to chemotherapy

Plasmodium vivax
"Benign tertian malaria"
Widely distributed, occurring in both tropical and temperate climates; chloroquine resistance has been reported in New Guinea

Plasmodium ovale
"Ovale tertian malaria"
Primarily in sub-Saharan Africa, where ecologically it replaces *P. vivax*

Plasmodium malariae
"Quartan malaria"
Can persist in blood for years

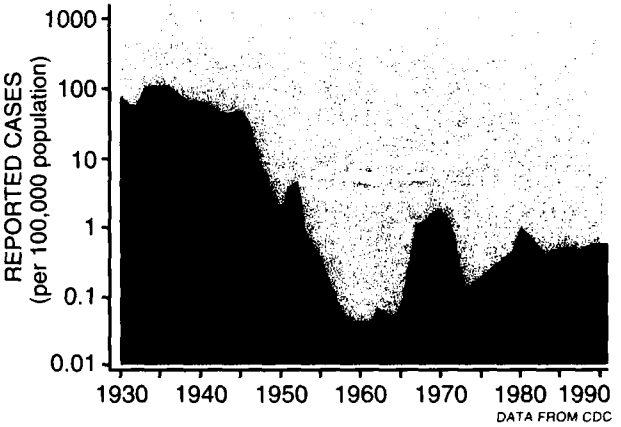
The importance of protective bed nets, understood in ancient Egypt, is taught today in Tanzania by means of a play. Here a mosquito, diverted by a bed net, preys upon an unprotected victim.

COURTESY OF CLIVE SHIFF, Ph D

Malaria in the United States

Plasmodium vivax malaria was transmitted in the southern states until World War II. Its eradication, based upon a combination of factors including drainage of swamps and liberal use of insecticides at breeding sites, was accomplished primarily by the general improvement in living conditions after the depression.

During and shortly after the war many cases of relapsing malaria occurred in troops returning from overseas. Malaria cases reported to the CDC progressively declined in numbers thereafter until the 1960's, except for 1950–51, when soldiers returned from Korea with *P. vivax*. A similar increase occurred between 1965 and 1971 in returning Vietnam veterans.



Currently about 1500 cases are reported each year, mostly among immigrants and travelers. In 1992 malarial parasitemia was detected in 178 of 376 Montagnard refugees being settled in North Carolina. *P. falciparum* and *P. vivax* were the most common species, but a few patients had *P. malariae* infections.

Although malaria transmission has been interrupted in the United States, the mosquito vectors are still present. Focal autologous transmission has occurred several times over the past 10 years in California and Texas. On each occasion parasitemic migrant laborers from endemic countries who were living outdoors or in inadequate housing infected local anopheline mosquitoes, which then in turn infected local inhabitants.

The potential also exists for malaria to be reintroduced, as has happened in Europe. Stowaway mosquitoes on airplanes coming from endemic countries have infected people living near international airports.

to carry the transgenic constructs throughout the wild population. The goal of this long-range control strategy is to combine several parasite-inhibiting genes into a single infectious vehicle, reducing the chances that the parasite will evolve resistance.

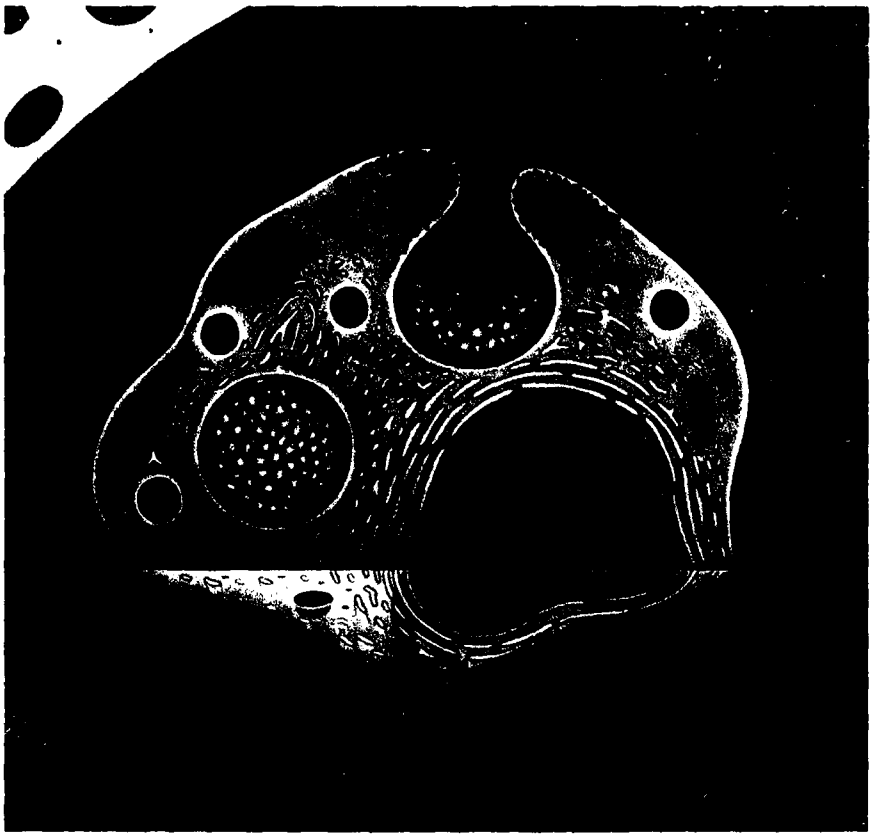
Drugs Encounter Parasite Resistance

The introduction of chloroquine in the 1940's brought great optimism for the control of malaria because the drug was cheap, highly effective, and well tolerated. That hope was short-lived. Chloroquine-resistant *P. falciparum* developed simultaneously in South America and Southeast Asia in the late 1950's and is now present in most malarious areas of the world. Chloroquine-resistant *P. vivax* has recently been discovered in Papua New Guinea and Indonesia. As other antimalarials have been introduced over the past 30 years, resistance to them has often followed closely.

The current situation is one of increasing resistance, often to multiple drugs, and a need for new and effective drugs. Discoveries about the mechanisms of action of antimalarials and resistance to them will aid in new drug development.

Resistance to chloroquine is the result of decreased accumulation of the drug in the parasite's food vacuole, either because of a transmembrane pump or because of an alteration in the gradient responsible for drug concentration. Verapamil, a calcium channel blocker, increases the accumulation of chloroquine within the vacuole and renders resistant parasites susceptible to chloroquine in vitro. Other methods of reversing chloroquine resistance are being studied.

Multiple drug resistance among *P. falciparum* also appears to result from the ability of the parasite to decrease intracellular concentrations of drugs. A proposed multiple drug resistance gene has been identified and sequenced; whether its presence predicts resistance is not clear.



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Hemoglobin is degraded to amino acids and heme in the food vacuole of the parasite's erythrocytic stage. Heme in soluble form is toxic to the parasite, which converts it into an insoluble form known as hemazoin (malaria pigment). Chloroquine acts by inhibiting the enzyme heme polymerase, critical for the conversion of heme to hemazoin. Soluble heme remains and kills the parasite.

Dihydrofolate reductase is required for the production of folic acid by malaria parasites, which make folic acid in large amounts. Pyrimethamine and proguanil inhibit this enzyme and are toxic to the parasite. Mutations in the dihydrofolate reductase gene are associated with altered binding affinity for the drugs and thus with drug resistance. Knowing the structural changes in the enzyme that are associated with resistance has allowed the synthesis of analogs that are more effective in animal and laboratory models.

Resistance to Fansidar (pyrimethamine and sulfadoxine) is also widespread. Quinine is still effective in most areas for chloroquine-resistant infections, but resistance to quinine is emerging in some areas, especially Thailand. Mefloquine, a 4-quinolinemethanol compound structurally related to quinine, was approved by the FDA in 1990 for chemoprophylaxis and therapy in areas with chloroquine-resistant falciparum malaria. Resistance to mefloquine is already prevalent along the borders of Thailand, and in these areas, doxycycline is the drug of choice for chemoprophylaxis. Halofantrine, approved by the FDA in 1992, is a phenanthrene-methanol with a short half-life, useful in treatment of chloroquine-resistant malaria but not in prevention.

A promising drug for the future is a compound derived from *Artemisia annua*, the sweet wormwood, known in China as qinghaosu and in the west as artemisinin. The drug is well tolerated and rapidly reduces parasitemia, even in patients with multi-drug-resistant parasites.

Vaccines Hold Promise

Immunization with live attenuated sporozoites or blood-stage parasites is not practical; but consider these facts:

• In areas with intense transmission of malaria, the prevalence, incidence, morbidity, and mortality of malaria decrease with increasing age; naturally acquired immune re-

ADVICE TO TRAVELERS

Malaria can be prevented in travelers to the few endemic regions where chloroquine resistance has not yet developed by prescribing 300 mg of chloroquine once a week, beginning before departure and continuing for four weeks after return. That amount of chloroquine base is contained in one 500 mg tablet of chloroquine phosphate (Aralen) or two 200 mg tablets of hydroxychloroquine sulfate (Plaquenil). For travel into areas where chloroquine resistance has been reported, one 250 mg tablet of mefloquine (Lariam) can be prescribed per week on the same schedule.

Alternative approaches exist. Travelers to areas where the parasites are still susceptible to the combination of pyrimethamine and sulfadoxine (Fansidar) can be given chloroquine prophylaxis and provided with three Fansidar tablets to take if symptoms of malaria develop. Particularly in East Africa, a combination of daily proguanil (Paludrine, which is not available in the United States), 200 mg, and weekly chloroquine, 500 mg of the salt, has been shown to provide partial protection. Finally, in areas with intensive transmission of multi-drug-resistant falciparum malaria, doxycycline, 100 mg daily, has been given with or without weekly chloroquine for short-term prophylaxis in travelers with heavy exposures.

Travelers exposed to *P. vivax* or *P. ovale* may require terminal prophylaxis with primaquine when they return home to prevent relapses from liver-stage infections.

The increasing prevalence of drug-resistant *P. falciparum* malaria makes it essential to stress the use of insect repellants, insecticides, and bed nets. Reduction of contact with feeding mosquitoes is the safest and most cost-effective means of preventing malaria in travelers.

sponses limit the impact of malaria.

- Purified IgG from West African adults has been used to successfully treat Thai children with multi-drug-resistant *P. falciparum*; anti-*P. falciparum* antibodies can have a dramatic effect on the parasite in humans.
- Immunization with radiation-attenuated *P. falciparum* sporozoites completely protects volunteers against experimental challenge for at least 9 months.

P. falciparum changes its antigenic characteristics during its life cycle, so that a successful vaccine must either completely eliminate or profoundly reduce parasite numbers at one stage of the life cycle or else attack the parasite at all stages. Some antibody and cellular mechanisms responsible for protective immunity have been identified. Important target antigens expressed at the sporozoite, liver, and asexual and sexual blood stages have been discovered.

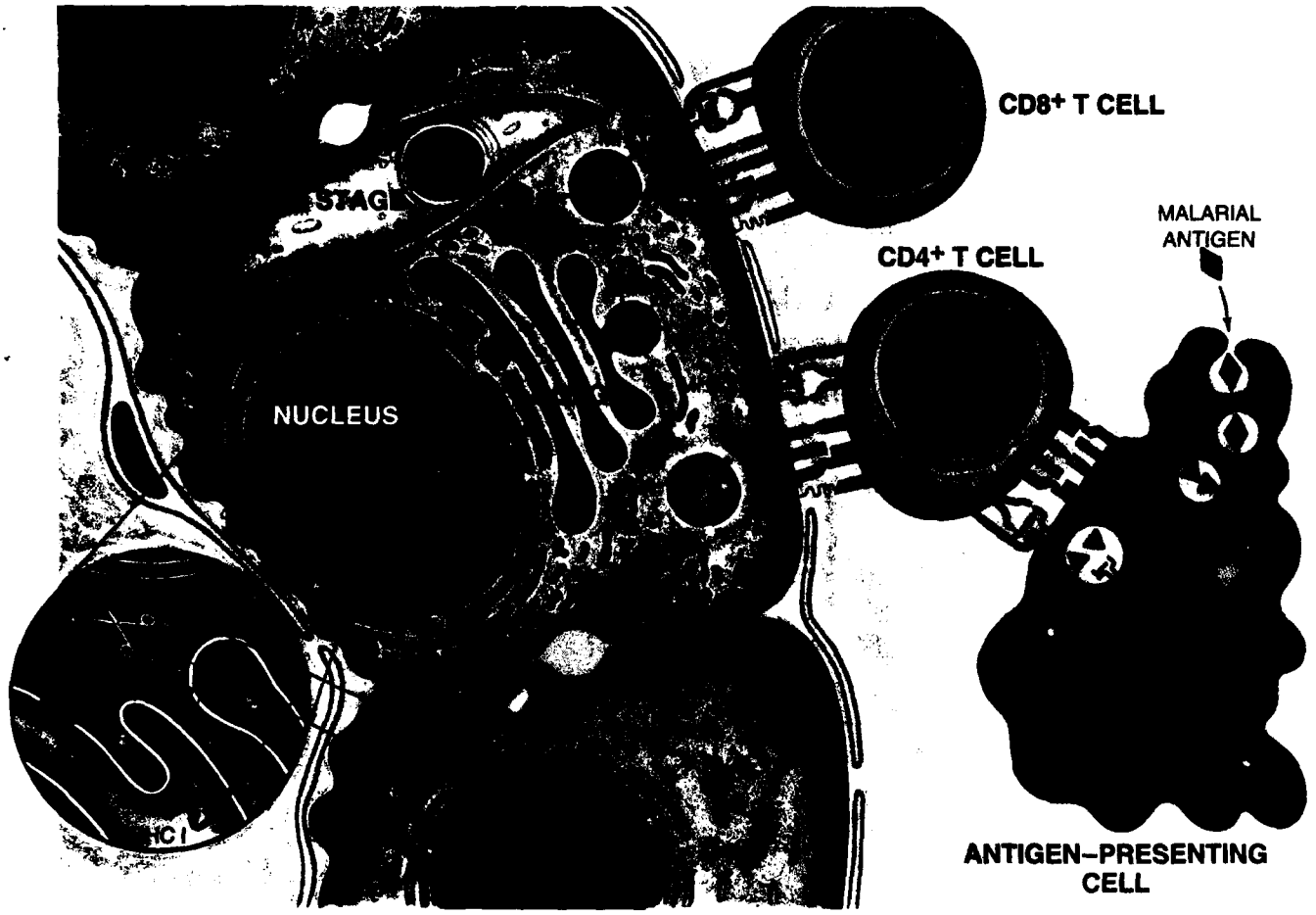
Sporozoites are in the circulation for less than an hour before entering the liver. Because they are extracellular, the only immune response that could prevent their invading hepatocytes is an antibody response. In 1980 researchers at New York University showed that passive transfer of monoclonal antibodies directed against the major surface protein of sporozoites, the circumsporozoite protein or CSP, could completely prevent infection of mice with *Plasmodium berghei*, a rodent malaria. Monoclonal antibodies against *P. vivax* CSP prevent infection in monkeys, and monoclonal antibodies against *P. falciparum* CSP can prevent in vitro invasion and development in human hepatocytes by *P. falciparum*. CSP's from different *Plasmodium* species all have a central region of tandemly repeated amino acids, and all the protective monoclonal antibodies are directed against these repeats.

More than 15 experimental synthetic peptide and recombinant DNA–produced purified protein vaccines targeting sporozoite surface proteins have been tested, and all have been shown to be safe. They have evoked varying levels of immunogenicity, and a number of them have given 15% to 25% protection against experimental challenge. A new generation of such vaccines constructed as branched-chain polymers, more immunogenic and protective in mice, will soon be tested in human volunteers with the traditional adjuvant aluminum hydroxide and new adjuvants such as liposomes and block copolymers.

A second protein called sporozoite surface protein 2 (SSP-2), discovered at the Naval Medical Research Institute, is the target of antibodies that can prevent malaria. CSP and SSP-2 have a region of homology that has been implicated as being important for sporozoite binding to hepatocytes and a suitable target for a vaccine.

Parasites developing within hepatocytes were long thought to be sequestered from the immune system. However, in the late 1980's mice that had been immunized with radiation-attenuated sporozoites and protected against sporozoite challenge were found to lose their immunity if their $CD8^+$ T cells were eliminated by in vivo treatment with monoclonal antibodies. Since irradiated sporozoites do not protect against challenge with infected erythrocytes, since T cells recognize peptides only in combination with major histocompatibility complex molecules on the surface of cells, and since sporozoites in circulation are extracellular, $CD8^+$ T cells must be recognizing parasite peptides presented by class I MHC molecules on the surface of infected hepatocytes.

Intravenous injection of $CD8^+$ cytotoxic T lymphocyte clones against CSP or SSP-2 completely protects normal mice against malaria infection, demonstrating that T cells can kill infected hepatocytes. Moreover, $CD4^+$ T cells against CSP are also protective in adoptive transfer, antibodies against a newly



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discovered liver-stage antigen of *P. yoelii* eliminate infected hepatocytes from culture, and cytokines such as interferon-γ can also prevent malaria, presumably by inducing the infected hepatocytes to produce nitric oxide that kills the parasite.

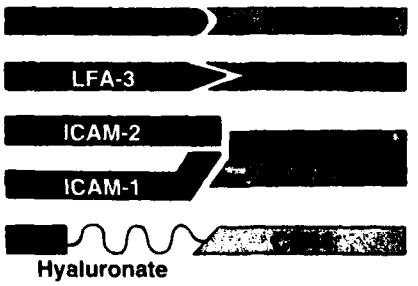
Naturally acquired $CD8^+$ CTL responses may also reduce morbidity and mortality from malaria. Among Gambian children there is a significant correlation between HLA-Bw53, a class I MHC molecule, and protection against severe malaria. HLA-Bw53–positive Gambians produce cytotoxic lymphocytes against a peptide from a *P. falciparum* protein called liver-stage antigen 1 (LSA-1).

Among vaccines designed to induce protective antibody and T cell responses against parasite proteins expressed in hepatocytes, one of the first to reach humans will be a recombinant vaccine expressing CSP, SSP-2, LSA-1, and four blood-stage antigens, developed by the Walter Reed Army Institute of Research and Virogenetics, Inc., in collaboration with scientists from numerous institutions in the United States and Europe.

The asexual erythrocytic stage of the parasite is responsible for the human disease. Antibodies that have been shown to provide passive protection against *P. falciparum* infection could recognize extracellular merozoites released from infected hepatocytes or infected erythrocytes and prevent them from invading erythrocytes, or they could recognize parasite antigens on the surface of infected erythrocytes and eliminate these cells from the circulation.

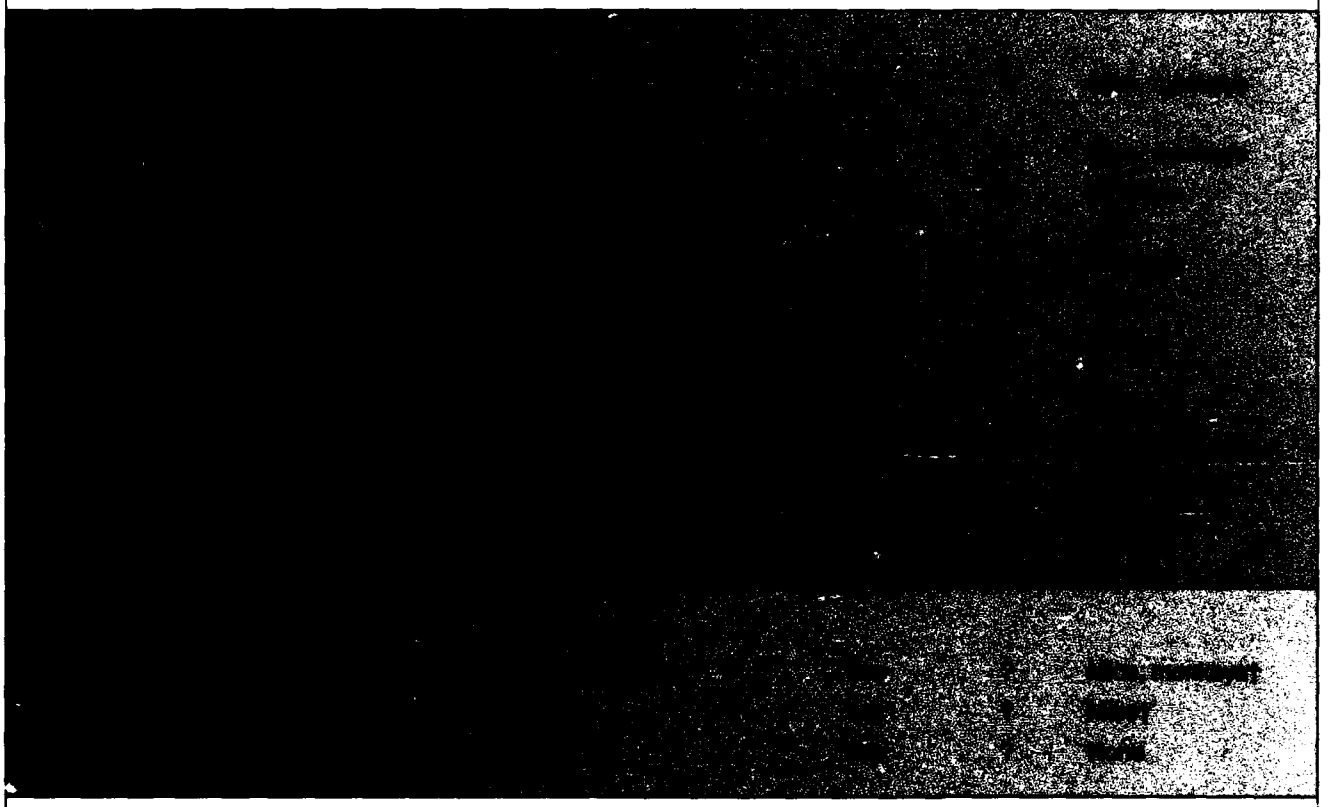
The severe manifestations of *P. falciparum* infection are due in part to microcirculatory obstruction caused by parasite antigens on the surface of infected erythrocytes sticking to capillary and postcapillary venule endothelium and to

Cellular responses to liver-stage *Plasmodium* infection are still being defined. As shown here schematically, they undoubtedly involve processing and presentation of the parasite by the hepatocyte and a complex set of receptor-ligand interactions between the infected hepatocyte and T lymphocytes:



TARGET PROTEINS FOR MALARIA VACCINE DEVELOPMENT

Antigen	Synonyms	Molecular Weight (kD)	Known Locations	Known Target of Protective Antibodies	Known Target of Protective T Cell Response	Host Protection as a Vaccine
PRE-ERYTHROCYTIC STAGES						
CSP	None	58	Sporozoite, hepatocyte	Yes	Yes	Mice, monkeys, humans
SSP-2	TRAP	90	Sporozoite, hepatocyte, RBC's	Yes	Yes	Mice
LSA-1	None	230	Hepatocyte	?	?	None



*Host antibody blocks merozoite invasion of RBC's in vitro.
†Host antibody blocks transmission in mosquitoes.

other erythrocytes (cytoadherence), and in part to host cellular products such as tumor necrosis factor that are released in response to stimulation of reticuloendothelial cells by parasite-derived soluble proteins. Antibodies could also prevent such effects by cross-linking parasite proteins, by recruiting complement to lyse the cells, or by recruiting cells such as macrophages to kill the parasite. The host could also induce T cells to release cytokines that either directly kill the parasite, induce erythrocytes to do so, or induce reticuloendothelial cells to secrete other parasiticidal cytokines. To be effective, T cells would have to be sensitized by antigen-presenting cells, since mature erythrocytes do not have MHC molecules on their surfaces.

Many parasite antigens expressed at the asexual erythrocytic stage have been characterized. The most promising are three merozo-

ite surface proteins, called MSP-1, MSP-2, and MSP-3, erythrocyte-binding protein 175 that is thought to be critical for parasite binding to erythrocytes, apical merozoite antigen 1, and SERA. Immunization with a purified recombinant rodent malaria MSP-1 has protected mice against malaria, and a recombinant form of MSP-2 has been tested for safety and immunogenicity in humans in Australia.

During the past year the field of malaria vaccine development has been excited by reports from Colombia of successful immunization with a peptide polymer vaccine called SPf66. This vaccine includes peptides from *P. falciparum* MSP-1 and two undefined *P. falciparum* proteins. When administered to Colombians in a placebo-controlled trial, it gave 34% protection against a first infection with *P. falciparum* and 39% protection against multiple infections within one year. The incidence of malaria in Colombia, however, is considerably lower than in sub-Saharan Africa and parts of Asia, and SPf66 is now being evaluated in Tanzania, Thailand, and The Gambia.

A vaccine that prevented the development of the parasite within the mosquito would provide no protection for an individual against malaria, but could have profound effects on transmission of malaria. Several proteins on the sexual stages of malaria parasites that could be targets for antiparasitic antibodies have been cloned and sequenced. A protein that is not present in gametocytes but first appears in gametes, called Pfs25, that was discovered at the NIH, is the most promising such target.

The ideal malaria vaccine will induce antibody and cellular immune responses against all stages of the parasite's life cycle. It will induce antibodies that block sporozoite invasion of hepatocytes, antibodies and T cell responses that kill infected hepatocytes, antibodies that block merozoite invasion of erythrocytes, antibodies and cytokines that kill infected erythrocytes, antibodies that prevent infected erythrocytes from adhering to endothelial cells, antibodies that prevent the release of harmful soluble parasite proteins, and antibodies that prevent the development of the parasite in mosquitoes.

Investigators throughout the world are drawing near to accomplishing each of these goals. They may soon have the opportunity to take advantage of astounding recent developments in vaccine design and delivery to construct completely new generations of multicomponent vaccines.

P. falciparum is a cunning animal that has kept several paces ahead of all drugs thrown at it. There is no reason to assume that it will not outwit vaccine-induced protective immune responses, no matter how complex. Nonetheless, the coming years will see the development and fielding of a series of vaccines designed to control this enormously important infection.

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Regulation of Myocardial Adaptation

by Larry Kedes

Along with the sequencing of human genes and the cloning of their protein products, there is considerable progress being made in the characterization of how genes are regulated. Specific DNA sequences, mostly in the untranslated regions of the genome, are bound to proteins, some of which are found in all cells and some of which are cell-type–specific. These interactions are signals that influence gene expression. Regulation of genes responsible for producing the contractile proteins of striated muscle has important clinical implications. Myocardial hypertrophy associated with dynamic overload results from alterations in the regulatory mechanism.

Only some of the 100,000 or more human genes are expressed at any given time. Those that encode proteins required in large amounts are busier than those whose products are less abundant. Gene expression is regulated by the binding of nuclear proteins to certain nucleotide sequences in DNA, which are the promoters and enhancers of gene expression.

The response of a gene to a signal that its expression should be changed, either increased or decreased, can take one of three forms. The modification of gene expression can persist for as long as the signal does, or it can be transient, or it can persist even after the signal ceases. This aspect of the regulation of gene expression applies to all genes and their protein products.

In addition, some encoded proteins are tissue-specific. Expression of these proteins gives certain cells their phenotypes. The contractile proteins of skeletal and cardiac muscle for example, are expressed only in these cells, though the genes that encode them are found in all cells. Some of these proteins are shared by the two muscle types, while others are not. Within the myocardium, there are differences between atrial and ventricular forms of the same protein and between fetal and adult forms as well, in some cases. These contractile proteins appear successively in a precise developmental sequence. Later forms can be replaced by earlier forms under the influence of environmental changes that may occur during life.

The notion that structurally different proteins, called isoforms, could have the same biologic function arose from studies that began in the 1960's. Michael Bárány of the Institute for Muscle Disease in New York correlated the speed of skeletal muscle contraction with the rate of myosin ATP hydrolysis in 1967, and skeletal myosin was found to have two kinds of subunits (the heavy and light chains) at about the same time. That the light chains were different in fast and slow muscle was established by Susan Lowey and Dennis Risby of Harvard in 1971.

Development of an electrophoretic method of analysing intact myosin in the laboratory of Joseph F. Y. Hoh at the University of Sydney led to the understanding of the structural differences among various forms of myosin in both skeletal and cardiac muscle. In 1981 a group led by Bernard Swynghedauw in Paris was able to link the distribution of the isoforms of myosin with the speed of myocardial contraction.

Tissue-specific proteins can be generated in various ways. Some contractile proteins are encoded by small families of genes (four to eight), and the molecular mechanism that selects a particular gene for expression in a particular cell is called selective transcription. Other proteins are produced in different forms by a single gene through a